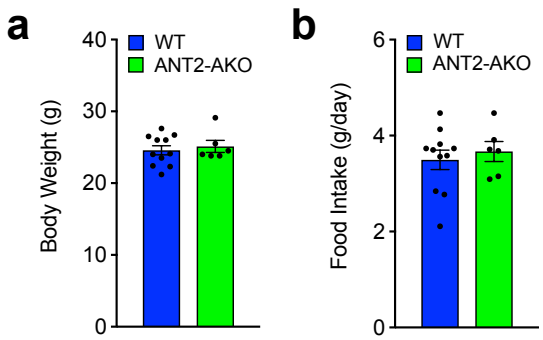
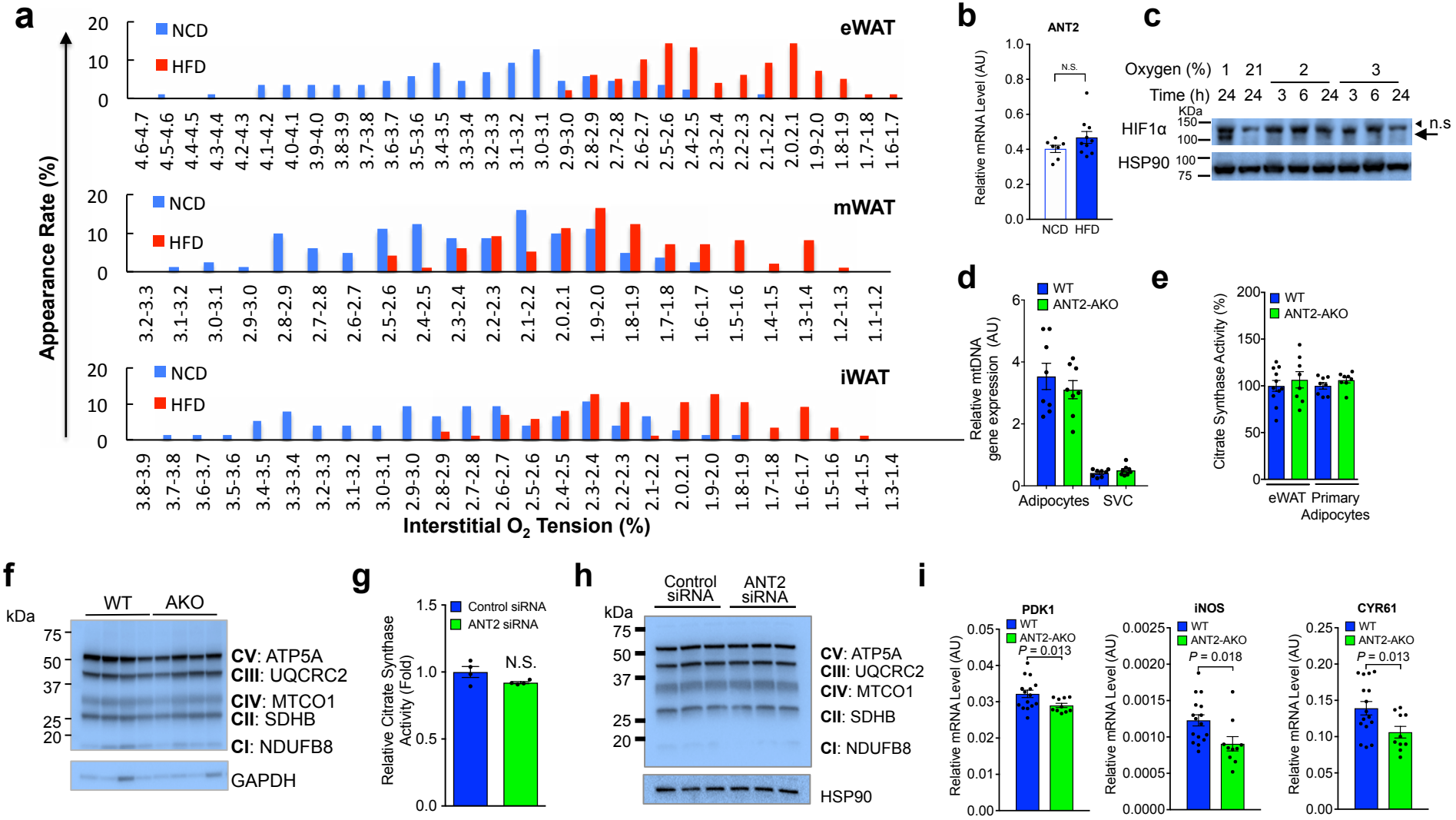
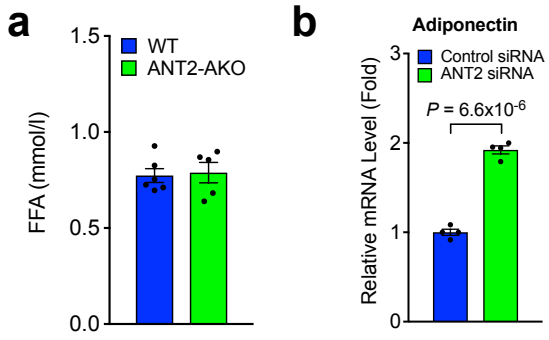


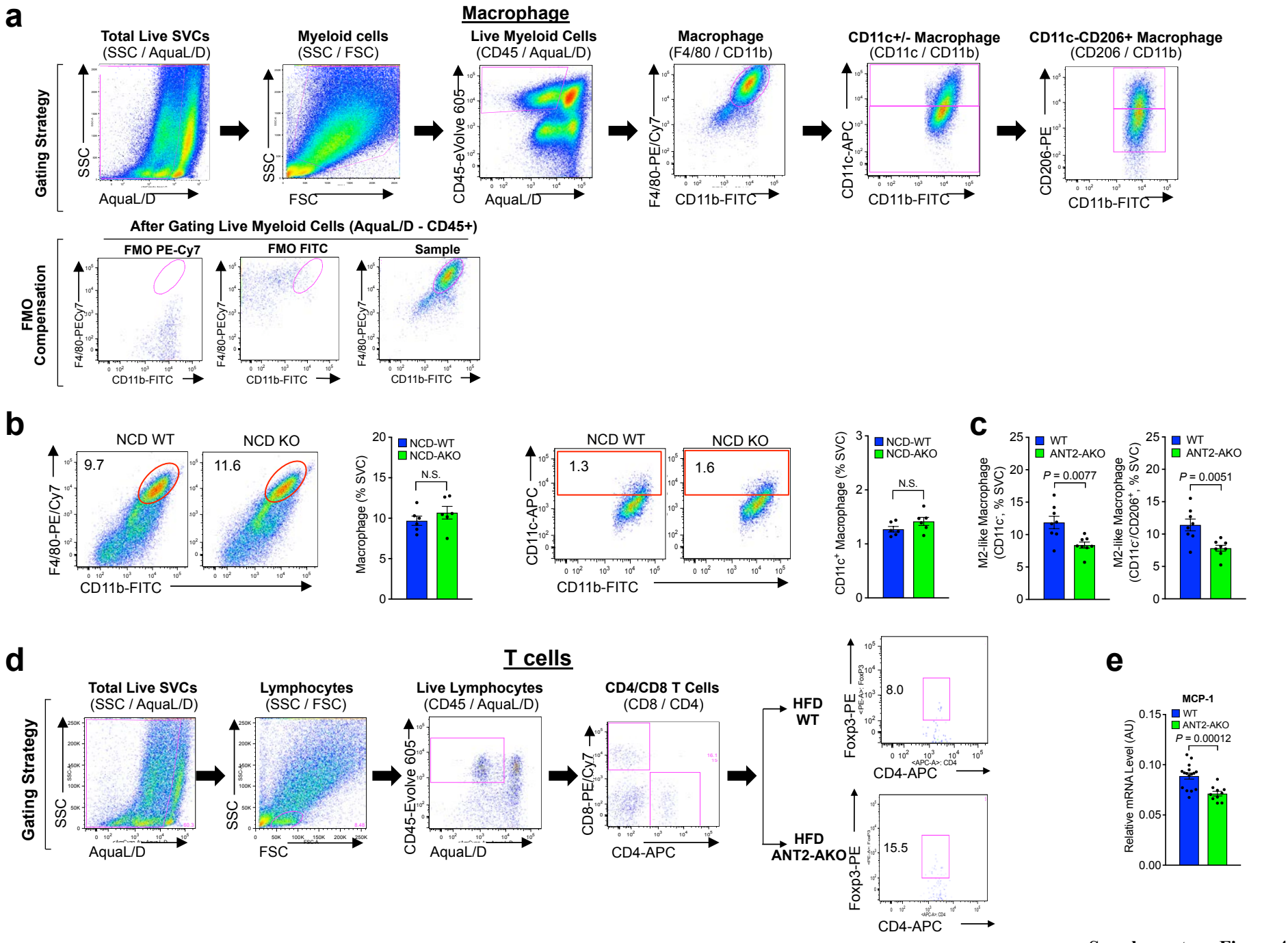
Supplementary Fig. 1 Body weight and food intake of NCD ANT2 AKO. (**a,b**) Body weight (**a**) and food intake (**b**) of WT (n=11 mice) and ANT2 AKO (n=6 mice) mice fed NCD. Data are presented as mean +/- SEM.



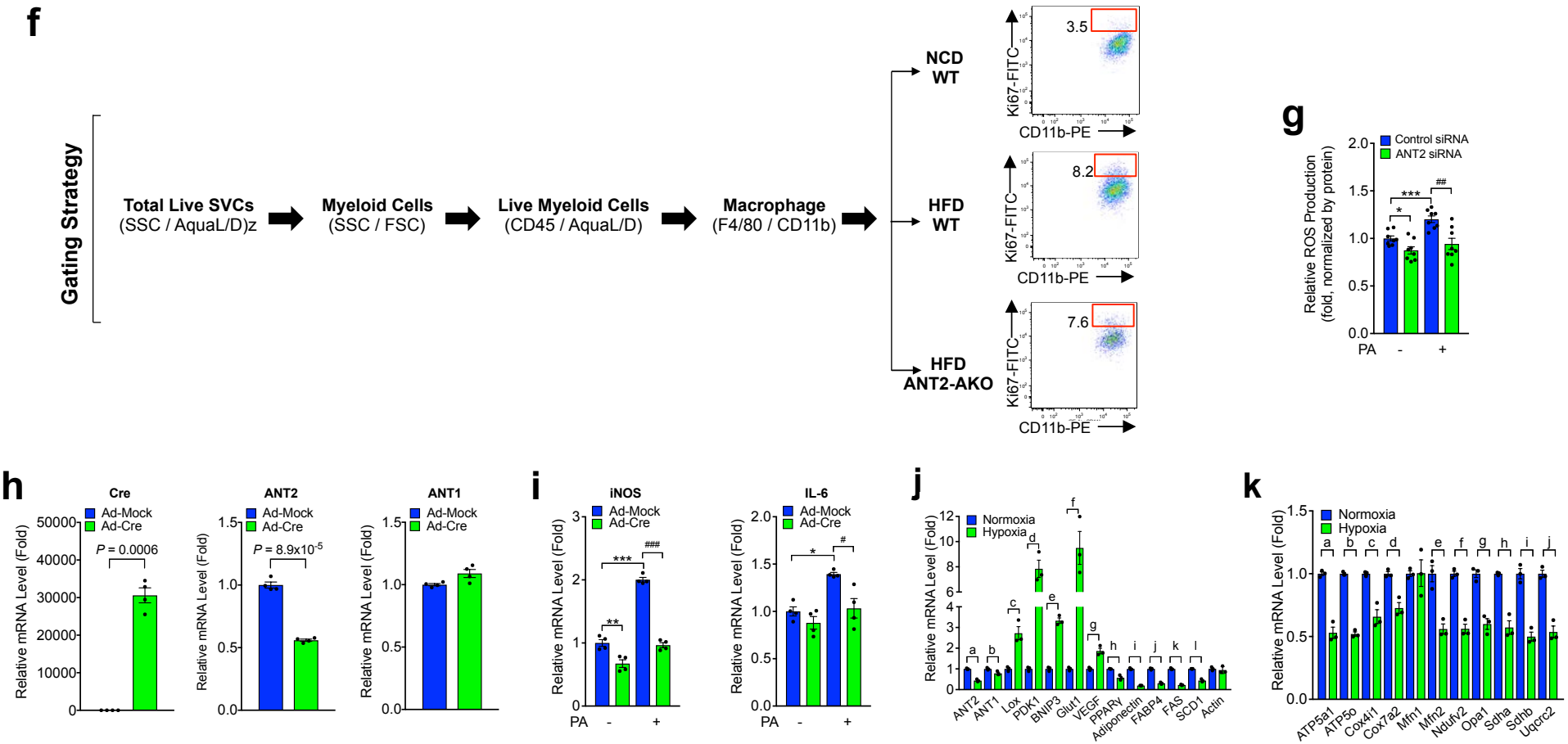
Supplementary Fig. 2 Interstitial adipose tissue O_2 tension in lean and obese mice and effect of ANT2 knockdown on CS activity and electron transport chain complex component expression in differentiated 3T3-L1 adipocytes. (**a**) Interstitial O_2 tension at different sites of eWAT, iWAT and mWAT in NCD and HFD WT mice (n=4 mice per group). (**b**) *Ant2* mRNA expression in NCD and HFD WT mouse eWAT (n=7 NCD and 8 HFD mice). N.S., not significant (*P*=0.122). (**c**) Western blot analysis of HIF-1α expression in 3T3-L1 adipocytes incubated at different O_2 conditions for 3, 6, or 24h. n.s., non-specific band. Similar results were obtained in at least two independent experiments. (**d**) Mitochondrial DNA content in primary adipocytes and SVC of WT and ANT2 AKO mice fed HFD (n=8 mice per group). (**e**) Citrate synthase activity in eWAT and primary adipocytes of WT and ANT2 AKO mice fed HFD (n=8 mice per group). (**f**) Western blot of analysis of OXPHOS mitochondrial complex components in primary adipocytes from HFD WT and ANT2 AKO mice. (**g,h**) Citrate synthase activity (**g**; n=4 mice per group) and expression of the electron transport chain complex components (**h**; n=3 mice per group) were measured in 3T3-L1 adipocytes transfected with control and anti-ANT2 siRNAs. N.S., not significant (*P*=0.147). Statistical analyses were performed by the two-tailed Student's *t* test. (**i**) mRNA levels of HIF-1α target genes in eWAT of HFD WT (n=16 mice) and ANT2 AKO mice (n=10 mice). In panels **b, g** and **i**, statistical analyses were performed by the two-tailed Student's *t* test. All data are presented as mean +/- SEM.



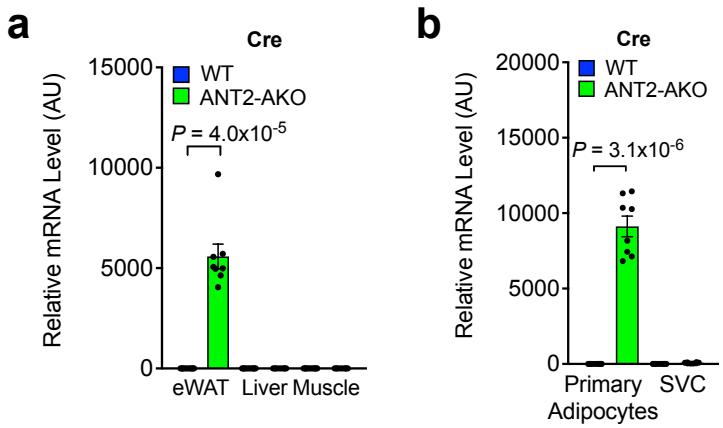
Supplementary Fig. 3 (a) Plasma FFA levels in 6h-fasted HFD WT (n=6 mice) or ANT2 AKO (n=5 mice) mice. Statistical analyses were performed by the two-tailed Student's *t* test. Data are presented as mean +/- SEM. (**b**) Adiponectin mRNA expression in control and ANT2 KD 3T3-L1 adipocytes (n=4 wells/ group). Statistical analyses were performed by the two-tailed Student's *t* test. Data are presented as mean +/- SEM.



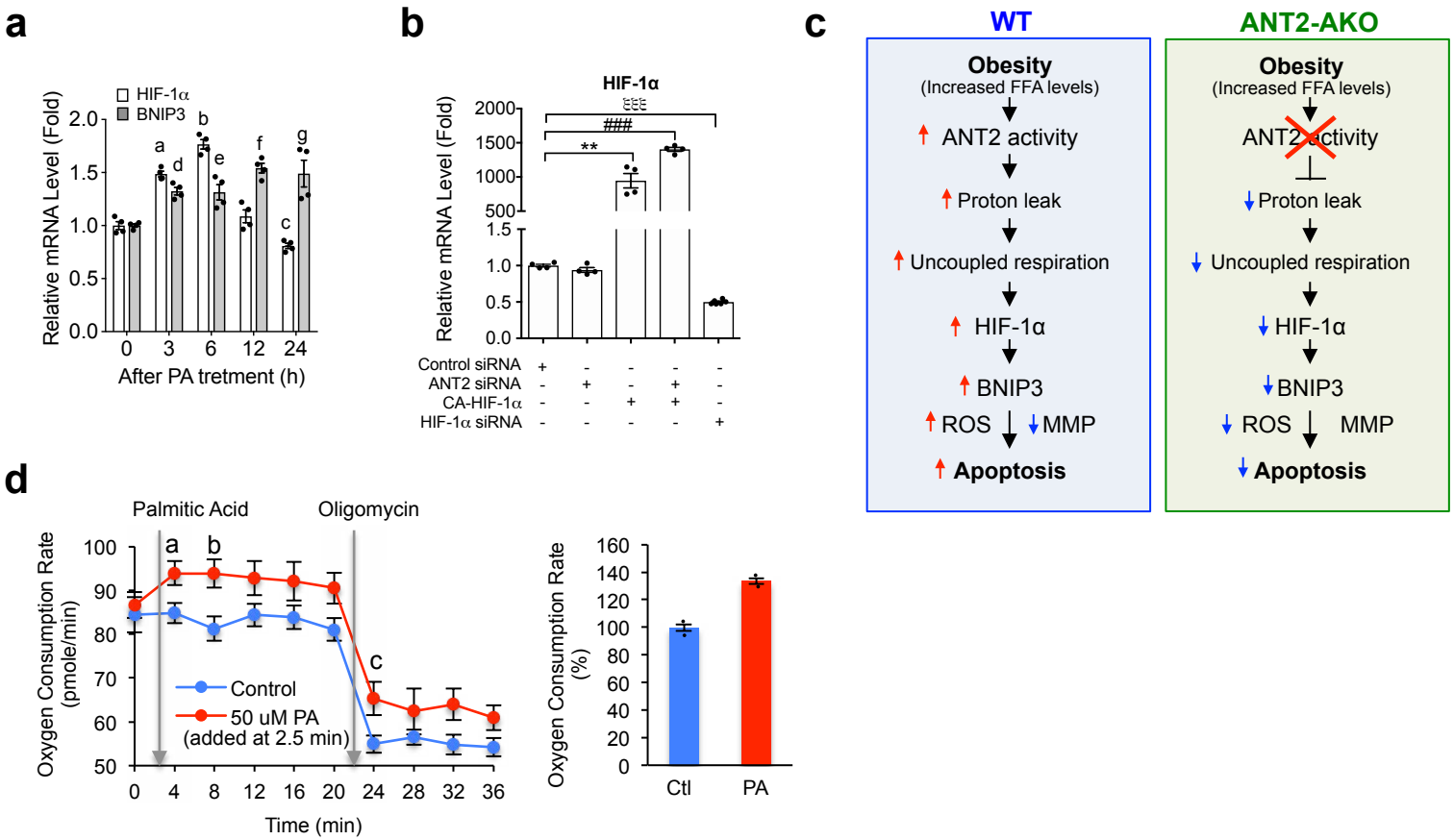
Supplementary Figure 4



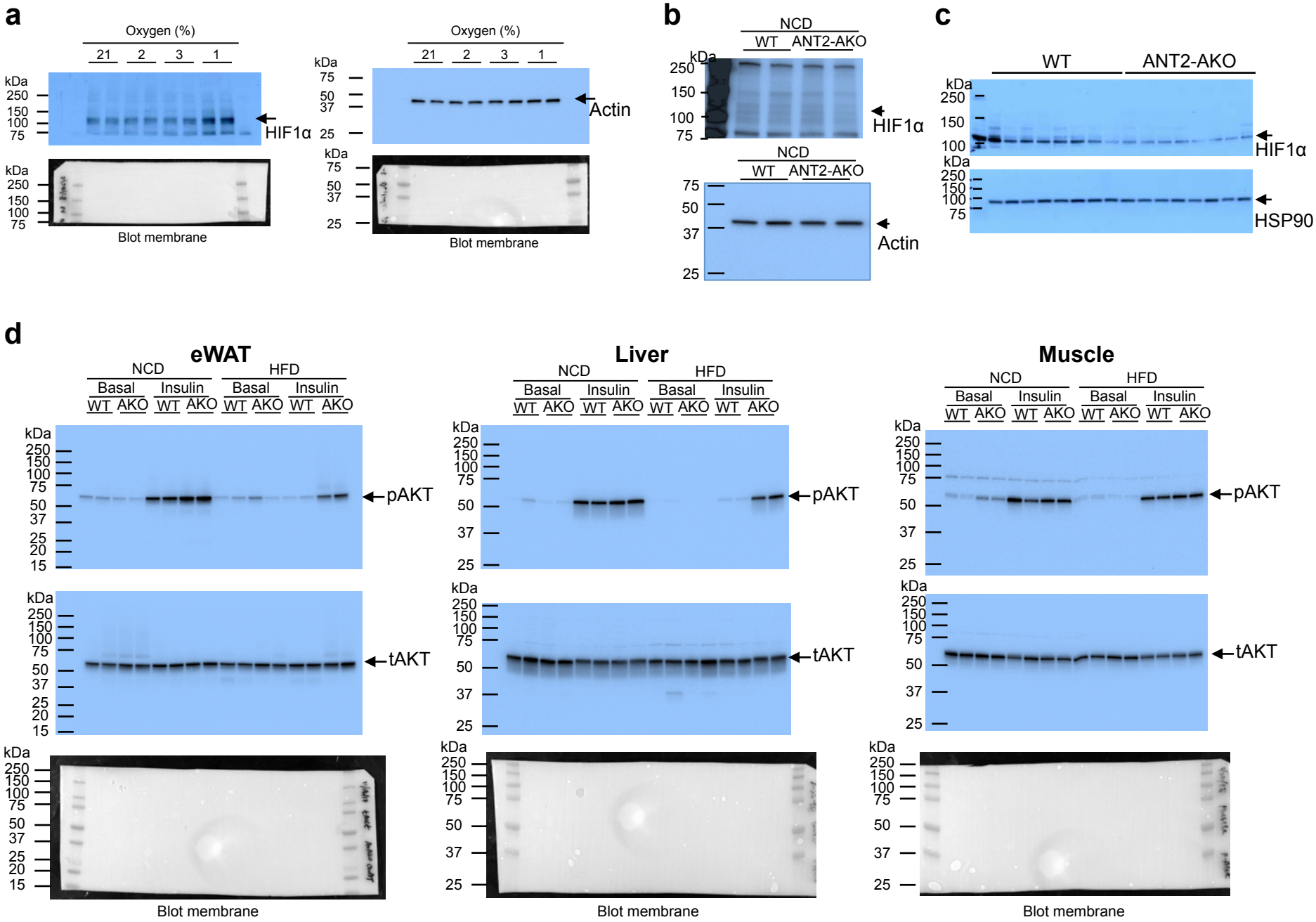
Supplementary Fig. 4 Flow cytometry analysis of ATMs of HFD WT and ANT2 AKO mice and inflammatory gene expression in WT and ANT2 KO adipocytes. (**a**) Upper panel: Gating strategy of the flow cytometry analysis of eWAT SVCs for macrophages. Bottom panel: FMO compensation. (**b**) Ratio of total or CD11c$^+$ ATM population in SVCs of NCD WT or ANT2 AKO eWAT (n=6 mice per group). N.S., not significant (left, *P*=0.331; right, *P*=0.145). Statistical analyses were performed by the two-tailed Student's *t* test. (**c**) M2-like polarized macrophages population in the eWAT of HFD WT and ANT2 AKO mice (*left*, CD11b$^+$ / F4/80$^+$ / CD11c$^-$; *right*, CD11b$^+$ / F4/80$^+$ / CD11c$^-$ / CD206$^+$) (n=8 mice per group). Statistical analyses were performed by the two-tailed Student's *t* test. (**d**) Gating strategy of the flow cytometry analysis of eWAT SVCs for Treg cells. (**e**) mRNA levels of MCP-1 in primary adipocytes from eWAT of HFD WT (n=16 mice) and ANT2 AKO (n=10 mice) mice. Statistical analyses were performed by the two-tailed Student's *t* test. (**f**) Gating strategy for the flow cytometry analysis of eWAT SVCs for Ki67$^+$ ATMs. (**g**) ROS levels in 3T3-L1 adipocytes transfected with control or anti-ANT2 siRNAs and incubated in the presence and absence of high palmitate media (PA; 400 mM) (n=8 wells of cells per group). **P*=0.019, ****P*=0.00047, ##*P*=0.0028. Statistical analyses were performed by the two-tailed Student's *t* test. (**h,i**) Effects of ANT2 deletion on inflammatory gene expression in primary adipocytes. Preadipocytes isolated from iWAT of ANT2$^{fl/fl}$ mice were differentiated into adipocytes by stimulating them with a hormonal cocktail. Control (Ad-Mock) or Cre expressing adenovius (Ad-Cre) was infected into these adipocytes, which was followed by incubation in no or high palmitate medium for 24 h (n=4 wells per group). mRNA expression of *Cre*, *Ant1* and *Ant2* (**h**) and *Nos2* and *Il6* (**i**) was measured by qRT-PCR. For *Nos2,* ***P*=0.0068, ****P*=1.1x10^{-5}, ###*P*=1.3x10^{-6}. For *Il6,* ***P*=0.0018, #*P*=0.037. Statistical analyses were performed by the two-tailed Student's *t* test. (**j,k**) mRNA expression in 3T3-L1 adipocytes incubated in normoxic (21% O_2) or hypoxic (1% O_2) conditions for 24h (n=3 wells per group). (**j**) ^aP=0.0082, ^bP=0.039, ^cP=0.029, ^dP=0.0094, ^eP=0.00083, ^fP=0.023, ^gP=0.016, ^hP=0.024, ^iP=0.00038, ^jP=5.0x10^{-5}, ^kP=4.1x10^{-5}, and ^lP=0.0038. (**k**) ^aP=0.0049, ^bP=0.00015, ^cP=0.022, ^dP=0.014, ^eP=0.0075, ^fP=0.0016, ^gP=0.023, ^hP=0.014, ^iP=0.00083, and ^jP=0.0025. Statistical analyses were performed by the two-tailed Student's *t* test. All data are presented as mean +/- SEM.



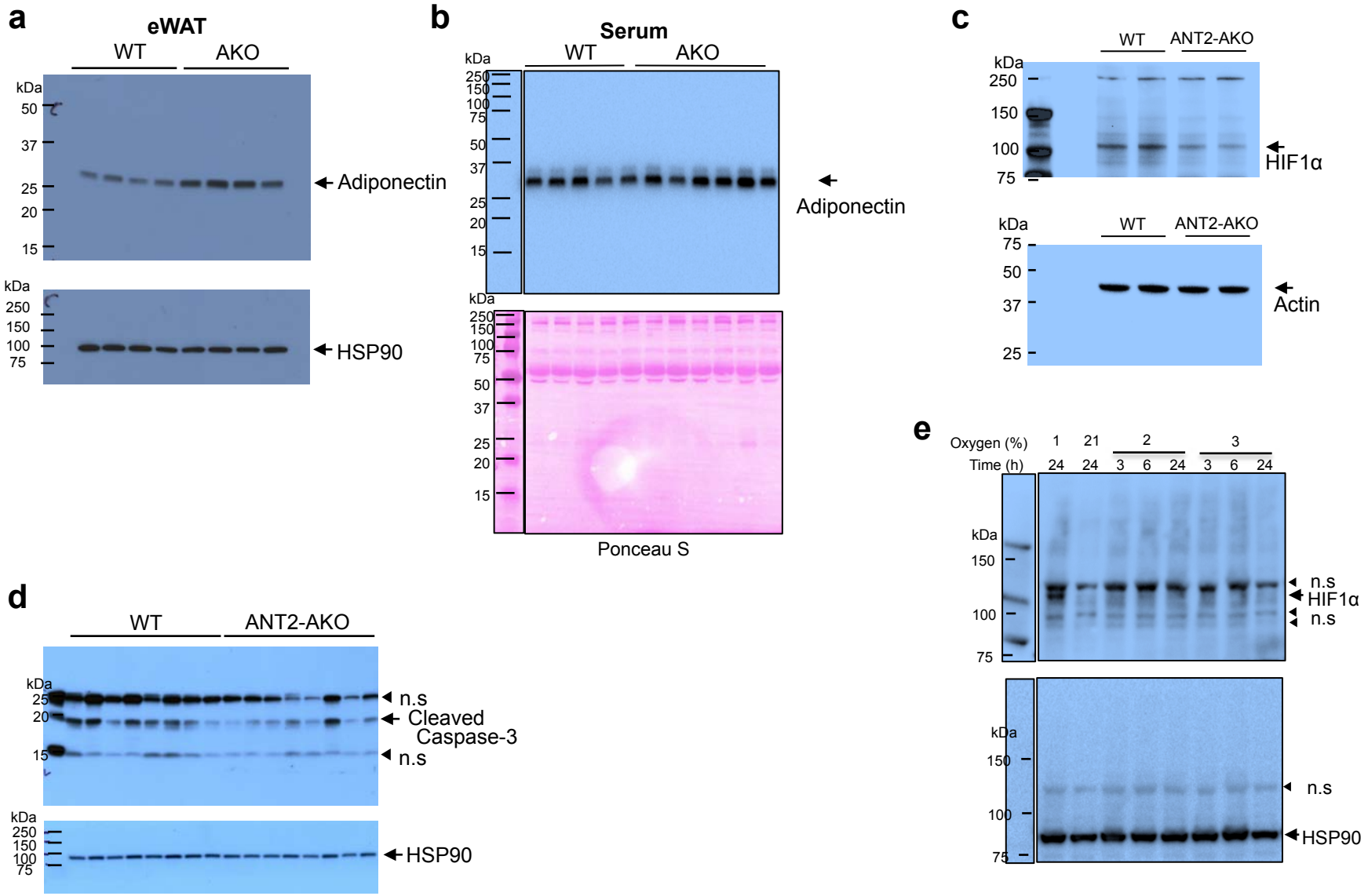
Supplementary Fig. 5 (a) Cre mRNA expression in eWAT, liver, or skeletal muscle of HFD WT (n=10) and ANT2 iAKO (n=8) mice. Statistical analyses were performed by two-tailed Student's *t* test. Data are presented as mean +/- SEM. (**b**) Cre mRNA expression in adipocyte or SVC fraction of eWAT of HFD WT (n=10) and ANT2 iAKO (n=8) mice. Statistical analyses were performed by two-tailed Student's *t* test. All data are presented as mean +/- SEM.



Supplementary Fig. 6 Effects of high FFA levels and ANT2 knockdown on the expression of adipocyte *Hif1a* and *Bnip3*. (**a**) mRNA expression *Bnip3* and *Hif1a* in 3T3-L1 adipocytes after high palmitate challenge (400 mM) (n=4 wells of cells at each time point). ^{a}P=7.4x10^{-5}, ^{b}P=1.4x10^{-5}, and ^{c}P=0.0068 vs lane 1; ^{d}P=0.0098, ^{e}P=0.020, ^{f}P=0.00058, and ^{g}P=0.029vs lane 2. Statistical analyses were performed by the ANOVA with post-hoc two-tailed Student's *t* test. (**b**) *Hif1a* mRNA expression in 3T3-L1 adipocytes transfected with constitutively active (CA) HIF-1α, anti-ANT2 siRNA and/or anti-HIF-1α siRNA. n=4,4,4,4, and 6 wells in lane 1 through 5. **P=0.0029, ###P=1.9x10^{-5}, and ^{ggg}P=1.2x10^{-6}. Statistical analyses were performed by the ANOVA with post-hoc two-tailed Student's *t* test. (**c**) Schematic representation of high FFA-induced adipocyte apoptosis. FFA stimulates ANT2-dependent uncoupled mitochondrial respiration by increasing proton leak through ANT2, causing state of relative hypoxia and increased HIF-1α expression. HIF-1α binds to *Bnip3* promoter and increases adipocyte apoptosis. (**d**) Oxygen consumption rate in 3T3-L1 adipocytes was measured before or 1.5 min after adding 50 mM palmitic acid (PA) or thereafter, or without PA treatment using Seahorse XF96 system (left panel) (n=6 wells per group). To measure more acute response to PA, 3T3-L1 adipocyte oxygen consumption rate was measured between 0 and 1 min after 50 mM PA treatment using Clark electrode chamber (right panel) (n=2 runs per group). ^{a}P=0.035, ^{b}P=0.043, ^{c}P=0.018. All statistical analyses were performed by the ANOVA with post-hoc two-tailed t-tests between the individual groups, and all data are presented as mean +/- SEM.



Supplementary Fig. 7 Raw Western blot images. Original uncropped scan images of Fig. 2g (**a**), Fig. 2h (**b**), Fig. 2j (**c**) and Fig. 3k (**d**) are shown here.



Supplementary Fig. 8 Raw Western blot data. Original uncropped scan images of Fig. 3m (**a**), Fig. 3n (**b**), Fig. 5f (**c**), Fig. 6b (**d**), and Supplementary Fig. 2c (**e**) are shown here.

Supplementary Table 1. Participant characteristics

	Metabolically Normal Lean (n=7)	Metabolically Normal Obese (n=11)	Metabolically Abnormal Obese (n=9)
Age (years)	35 ± 4	34 ± 2	43 ± 2*†
Sex (Male/Female)	3/4	1/10	2/7
Body mass index (kg/m^2)	23.4 ± 0.6	36.4 ± 1.2*	38.8 ± 1.6*
Body fat (%)	29.8 ± 2.0	47.2 ± 2.2*	48.4 ± 2.0*
Fasting glucose (mg/dL)	84 ± 2	87 ± 1	103 ± 5*†
2-h OGTT glucose (mg/dL)	94 ± 9	113 ± 5*	173 ± 9*†
HbA1c (%)	5.0 ± 0.2	5.1 ± 0.1	5.8 ± 0.3*†

Data are means±SEM. OGTT: oral glucose tolerance test. ANOVA revealed a significant main effect of group for age ($P = 0.034$), body mass index ($P < 0.001$), body fat ($P < 0.001$), fasting glucose ($P < 0.001$), 2-h OGTT glucose ($P < 0.001$) and HbA1c ($P = 0.036$). Tukey-Kramer post-hoc analysis revealed the following significant differences:*Value significantly different from corresponding value in the Metabolically Normal Lean group, $p < 0.05$. †Value significantly different from corresponding value in the Metabolically Normal Obese group, $p < 0.05$.

Supplementary Table 2. Quantitative Realtime RT-PCR Primer Sequences.

Gene full name		Primers used for real-time QPCR	
		Forward	**Reverse**
Actin	Beta-acin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATG
Adiponectin	Adiponectin, also known as AdipoQ and Acrp30	TGTTCCTCTTAATCCTGCCCA	CCAACCTGCACAAGTTCCCTT
ANT1	Adenine nucleotide translocase 1	TCAAGTCGGACGGCCTG	CACCAGCCCCGCCACCGCT
ANT2	Adenine nucleotide translocase 2	TGCTGTCGCTGGCCTGACT	TGCGCCCTTGAAAAAAGCC
ATP5a1	ATP synthase F1 subunit alpha	TCTCCATGCCTCTAACACTCG	CCAGGTCAACAGACGTGTCAG
ATP5O	ATP synthase subunit O	TCTCGACAGGTTCGGAGCTT	TTGACGGTGCGCTTGATGTAG
BNIP3	Bcl-2/adenovirus E1B 19kD-interacting protein 3	ACTCAGATTGGATATGGGATTGG	GAGACAGTAACAGAGATGGAAGG
CD11b	Cluster of differentiation molecule 11B	TGGCCTATACAAGCTTGGCTTT	AAAGGCCGTTACTGAGGTGG
CD11c	Integrin alpha-X protein	CTGGATAGCCTTTCTTCTGCT	GCACACTGTGTCCGAACTC
Col1A1	Collagen type I alpha 1	GTGCTCCTGGTATTGCTGGT	GGCTCCTCGTTTTCCTTCTT
Col3A1	Collagen type III alpha 1	GGGTTTCCCTGGTCCTAAAG	CCTGGTTTCCCATTTTCTCC
Cox4i1	Cytochrome c oxidase subunit 4I1	ATTGGCAAGAGAGCCATTTCTAC	TGGGGAAAGCATAGTCTTCACT
Cox7a2	Cytochrome c oxidase subunit 7A2	GCTGGCCCTTCGTCAGATT	GGCATCCCATTATCCTCCTGAA
Cre	Cre recombinase	GCATTACCGGTCGATGCAACGAGTG	GAACGCTAGAGCCTGTTTTGCACGTTC
CYR61	Cysteine-rich angiogenic inducer 61	GGATGAATGGTGCCTTGC	GTCCACATCAGCCCCTTG
Elastin	Elastin	TGGTATTGGTGGCATCGG	CCTTGGCTTTGACTCCTGTG
F4/80	F4/80, aslo known as EMR1 and Ly71	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG
FABP4	Fatty acid binding protein 4	GGATTTGGTCACCATCCGGT	CCAGCTTGTCACCATCTCGT
FAS	Fatty acid synthase	AGTTCACGGACATGGAGCACAACA	ATGGTACTTGGCCTTGGGTGTGTA
Fibronectin	Fibronectin	CGAGGTGACAGAGACCACAA	CTGGAGTCAAGCCAGACACA
Glut1	Glucose transporter 1	CCTGTCTCTTCCTACCCAACC	GCAGGAGTGTCCGTGTCTTC
HIF-1α	Hypoxia-inducible factor 1-alpha	CAAGATCTCGGCGAAGCAA	GGTGAGCCTCATAACAGAAGCTTT
HIF-2α	Hypoxia-inducible factor 2-alpha	TAAAGCGGCAGCTGGAGTAT	ACTGGGAGGCATAGCACTGT
IL-6	Interleukin 6	GCTACCAAACTGGATATAATCAGGA	CCAGGTAGCTATGGTACTCCAGAA
iNOS	Inducible nitric oxide synthase	CTCAGCCCAACAATACAAGAT	TGTGGTGAAGAGTGTCATGCA
Lox	Lysyl oxidase	CCACAGCATGGACGAATTCA	AGCTTGCTTTGTGGCCTTCA
MCP-1	Monocyte chemoattractant protein 1	AGGTCCCTGTCATGCTTGTG	TCTGGACCCATTCCTTCTTG
Mfn1	Mitofusin 1	ATGGCAGAAACGGTATCTCCA	GCCCTCAGTAACAAACTCCAGT
Mfn2	Mitofusin 2	AGAACTGGACCCGGTTACCA	CACTTCGCTGATACCCCTGA
MIP1α	Macrophage inflammatory protein 1-alpha	CCAAGTCTTCTCAGCGCCAT	GAATCTTCCGGCTGTAGGAGAAG
Ndufv2	NADH:Ubiquinone Oxidoreductase Core Subunit V2	GCAAGGAATTTGCATAAGACAGC	TAGCCATCCATTCTGCCTTTG
Opa1	Mitochondrial dynamin like GTPase	TGGAAAATGGTTCGAGAGTCAG	CATTCCGTCTCTAGGTTAAAGCG
PAI-1	Plasminogen activator inhibitor-1	CATGCCCCACTTCTTCAAGCT	TGGTATGCCTTTCCACCCAGT
PDK1	Pyruvate Dehydrogenase Kinase 1	GGCCAGGTGGACTTCTATGC	AGCATTCACTGACCCGAAGT
PPARγ	Peroxisome proliferator activated receptor gamma	TCGCTGATGCACTGCCTATG	GAGAGGTCCACAGAGCTGATT
Rantes	Regulated on activation, normal T cell expressed and secreted	GCAAGTGCTCCAATCTTGCA	CTTGGCGGTTCCTTCGAGT
SCD1	Stearoyl-CoA desaturase 1	CCACCGCTCTTACAAAGCTC	CACGAGCCCATTCATAGACA
Sdha	Succinate dehydrogenase complex flavoprotein subunit A	GGAACACTCCAAAAACAGACCT	CCACCACTGGGTATTGAGTAGAA
Sdhb	Succinate Dehydrogenase Complex Iron Sulfur Subunit B	ATTTACCGATGGGACCCAGAC	GTCCGCACTTATTCAGATCCAC
TNFα	Tumor necrosis factor alpha	CATCTTCTCAAAATTCGAGT	TGGGAGTAGACAAGGTACAA
Uqcrc2	Ubiquinol-cytochrome c reductase core protein 2	AAAGTTGCCCCGAAGGTTAAA	GAGCATAGTTTTCCAGAGAAGCA
VEGF	Vascular endothelial growth factor	TACCTCCACCATGCCAAGTGGT	AGGACGGCTTGAAGATGTAC
36B4	60S Acidic Ribosomal Protein P0	GGCCCTGCACTCTCGCTTTC	TGCCAGGACGCGCTTGT
		Primers used for mtDNA content assays	
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase	GGCTCCCTAGGCCCCTCCTG	TCCCAACTCGGCCCCCAACA
mtDNA	Mitochondrial DNA	CCCAGCTACTACCATCATTCAAGT	GATGGTTTGGGAGATTGGTTGATGT